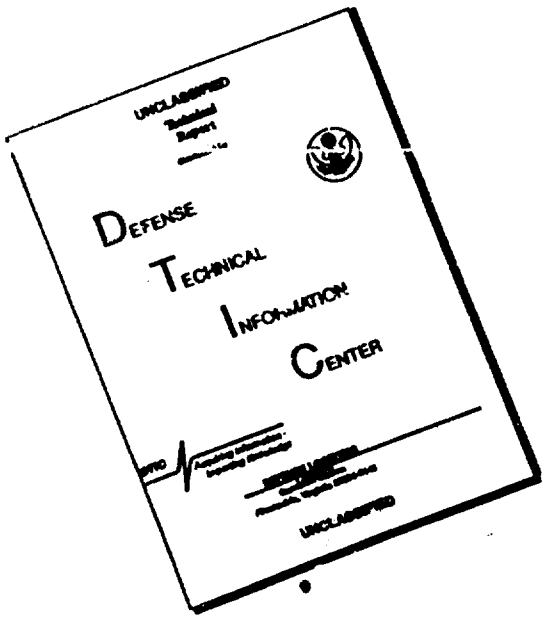
TRANSLATION NO. 95

DATE: Sept 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

Journal of Microbiology, Epidemiology and Immunobiology. No. 7: 64-69, 1955.

Cultivation of virus of spring-summer and Japanese encephalides on developing chicken embryo.

Andzhaparidze, O. G. (From the Laboratory of Virus encephalides; Headed by A. K. Shubladze, Institute of Virology AMS USSR; Director-P. I. Kosyakov.)

In this report we will set forth data on the cultivation of viruses of spring-summer and Japanese encephalides in developing chicken embryo.

At the beginning of our studies the question of cultivating the virus of Japanese encephalitis was very insufficiently illuminated in literature. Haagen and Krodel (1938) cultivated the virus on the chorionallantois membrane of an embryo of 14 days incubation. After 75 passages the virulence of the virus for white mice during this was not lowered, the virus became localized not only in the chorionallantois membrane, but also in the organs of the embryo. Smith and Lennet (1939), while comparing the activity of multiplication of the viruses of St. louis and Japanese encephalitis in chicken embryo, established that both viruses cause similar afflections to the chorionallantois membrane and in the brain of the chicken embryo and that the degree of multiplication of them in an egg is quite identical. Sebin and co-authors(1943) tried to utilize the culture of the Japanese encephalitis virus in developing chicken embryo as the initial material for the preparation of a vaccine, but, according to their data, the immunogenic properties of such a vaccine were very low.

The cultivation of the virus of spring-summer encephalitis was first conducted by Chumakov (1944), who established that the virus could be cultivated on the chorionallantois membrane of embryos of 8-12 days age.

During infection of chicken embryo by application of the virus onto the chorionallantois membrane, the maximum virus was detected not in the membrane or in the internal organs, nor even in the fluid of the embryo, but in the brain. There are no other data on the cultivation of the virus of spring-summer encephalitis in literature except those mentioned works of Chumakov.

In the tests of cultivation we utilized virus of Japanese (strain 7) and spring-summer encephalitis (strain Sofin). For the initial infection of the eggs we used a suspension of a fresh passage of mice brain, and in further passages- the virus-containing tissue of the infected embryo.

The titers of the virus of Japanese encephalitis during titration on mice was 10-6 to 10-6.5, virus of spring-summer encephalitis- 10-6.5 to 10-7.

For the infection we used live active embryo eggs with well developed blood vessels. All the manipulations during the cultivation on the embryo were conducted in strict aseptic conditions with sterile instruments. All the material for the passages and titration was subjected to an accurate bacteriological control by sowing in bullion, on semiliquid and oblique agar.

The infecting of the embryos was conducted by various methods: on the chorionallantois membrane, into the allantois cavity, into the yolk sac and in the cavity of the amnion. Methods of infecting have been discribed in literature extensively and therefore, we will not consider them here. During all methods of infecting the embryo, the virus-containing suspension was introduced in a volume of 0.05 ml. The infected eggs were placed into an incubator. After the incubation the contents of the eggs were extracted into sterile Kokh vessels, and then the required sections

of the embryo were taken with sterile instruments. In those cases when the distrubution of the virus in the sections of the eggs was being taken under study, the fluid was drawn off with a Pasteur pipet seperately; the embryo and the membrane were also drawn off seperately and successively washed in three Petri vessels with a sterile physiological solution. For passages on the embryo, a suspension of the membrane or foetus was taken, introduced in a dilution of 10-2 or 10-3.

We conducted a number of tests to establish the optimal conditions of cultivating the virus of Japanese and spring-summer encephalitis in developing chicken embryos.

In preliminary studies it became apparent that the virus of the spring-summer encephalitis is very pathogenic for chicken embryo and causes their death 5-6 days after infection. In successive tests we determined the optimal conditions of development of this virus in embryos: period of incubation, age, temperature of incubation of the infected embryos, quantity of the infecting dose of virus and path of infection. Having obtained the tentative data, we conducted a basic series of passages of the virus and again repeated all the tests, the results of which are below.

In order to determine the most advantageous period of incubation, we infected 9-10 day embryos by applying the virus to the chorionallantios membrane and placed them under 37 C. After 48, 72 and 96 hours of incubation the embryos were opened and a suspension from the tissues of the foetus and membranes was prepared for titration. As Table 1 shows, the most advantageous period of incubation for the cultivation of the virus of spring-summer encephalitis im edeveloping chicken embryos is 72 hours, although during other periods the virus multiplies and attains a significant concentration in the embryos.

The period of incubation for the virus of Japanese encephalitis was established by infecting the embryos in the yolk sac. During this method of infecting the greatest pathogenicity of the virus was observed, most of the embryos dying after 72 hours after infection. This test, as well as the succeeding ones, on the cultivation of the virus of Japanese encephalitis was conducted on a scheme which was analogical with the scheme of the tests with the virus of spring-summer encephalitis. The virus-containing suspensions were introduced into the yolk sac of 8-day incubated eggs. From them there were conducted 3 passages each of a suspension of the yolk membrane into the yolk sac in various periods of incubation- after 24, 48, and 72 hours. The infected eggs were incubated at 35.5 - 36 C. In all cases a suspension of the foetus and embryo membrane was used for titration. As Table 1 shows, the incubation of the infected embryos for 48 hours insures the greatest accummulation of the virus of Japanese encephalitis.

In the next series of tests we established the influence of the age of the embryo on the cultivation of both viruses. For the cultivation of the virus of spring-summer encephalitis we used embryos of 5, 7, 9, 10, 11, 12, and 15 day incubation. The embryos were infected in the chorionallantois membrane with a passage suspension of the chorionallantois membrane and incubated at 37 C. for 72 hours. Talbe 2 indicates the results of these tests. It became apparent that the 9-11 days embryos were the most suitable for the cultivating of the virus of spring-summer encephalitis.

With the virus of Japanese encephalitis the tests were conducted according to an almost identical scheme, but under different conditions: the temperature of incubation was 35.5 C, period of incubation was 48

hours and infected in the yolk sac. Under these conditions we tested embryos of 7, 8, 9, 11 days age. Table 2 shows that the virus in the said case cultivated better in the embryos of 8-9 days age.

To establish the best temperature of incubation, we infected embryos of 10 days incubation in the chorionallantois membrane with the virus of spring-summer encephalitis: one group of them was then incubated at 35.5, one at 37 and one at 39 C. 72 hours after infection the embryos were dissected and material was taken for titration. From the data set in Table 3 it is clear that the virus of spring-summer encephalitis cultivates best of all at 37 C.

Table 3 illustrates the comparative tests conducted with the virus of Japanese encephalitis. For this embryos of 9 days incubation were infected with a virus suspension in the yolk sac and incubated at 35.5, 37, and 39 C for 48 hours. After incubation the infected embryos were dissected and a suspension of the tissues of the foetus and membrane was prepared and used for titration. It appeared that the virus of Japanese encephalitis cultivates somewhat better at 35.5 C.

The intensity of cultivation of both viruses in chicken embryos depends to a significant degree on the path of infection of the latter.

In Table 4 are the results of cultivating the virus of spring-summer encephalitis in developing chicken embryos during infection through the chorionallantois membrane, into the allantois cavity, in the amnion and in the yolk sac. In order to determine the quantity of virus we took a collectivized suspension of tissues of the foetus and membrane. During infection by applying the virus on the chorionallantois membrane and into the yolk sac, the greatest accummulation of the virus of spring-summer encephalitis was noted; the virus of Japanese encephalitis cultivated best during infecting of the yolk sac.

In the next series of tests we studied the influence of the quantity of virus introduced during infecting of the chicken embryo on the intensity of its multiplication. For this we infected embryos with virus-containing suspensions in dilutions of 10-1, 10-2 and 10-4. For the cultivation of the virus, the optimal paths of infection, temperature, period of incubation and age of embryo which had been established by us earlier were used. The data of the tests with both types of virus indicated an absence of dependence between the quantity introduced and the abundance of it in the infected embryo.

Successive tests were applied for the establishment of the distribution of the virus in the infected chicken embryo. The passage suspension of virus of spring-summer encephalitis was introduced onto the chorion-allantois membrane of 9-day embryos and incubated at 37 C for 72 hours. The greatest quantity of virus was detected in the brain(10-6.7) and body of the embryo (10-6.3), in lesser(diminishing) quantities in the yolk membrane, chorionallantois membrane, amniotic and allantois fluid.

During cultivation of the virus of Japanese encephalitis by infecting the yolk sac of embryos of 9 day incubation and incubating them for 48 hours at 35.5 C, the virus was distributed in the embryos the same as with the virus of spring-summer encephalitis: the greatest quantity in the brain (10-6.7) and body (10-5) and further, in diminishing quantities, in the yolk and chorionallantois membrane and allantois fluid.

We conducted more than 30 passages of the virus of spring-summer encephalitis and 14 passages of the virus of Japanese encephalitis. The infected embryos were incubated in conditions which had been established by us as being optimal. During the passages the titers of the viruses were stable and equaled for the virus of spring-summer encephalitis 10-6 to 10-6.5, and for the viru of Japanese encephalitis 10-5 to 10-5.5.

No variations were noted in the embryos infected with the virus of Japanese encephalitis under microscopy, and in the embryos infected with the virus of spring-summer encephalitis there was observed dimness and oedema of the chorionallantois membrane, often oedema and ictericity of the embryo itself.

The virus of spring-summer encephalitis of the 29th passage and of Japanese encephalitis of the 14th passage were identified in cross-reactions of neurtralization with conforming immune serums.

CONCLUSIONS

1. The possibility of successfully cultivating the virus of spring-summer and Japanese encephalitis in developing chicken embryo was established.

2. The optimal conditions during cultivation of the virus of Japanese encephalitis are: 8-9 day embryos, infected in the yolk sac and incubated at 35.5 C for 48 hours. During such conditions of cultivation the virus is distributed in the eggs as follows: most in the brain of the embryo, then in the body of the embryo, in the allantois fluid, in the chorionallantois membrane and the least in the yolk membrane.

3. The optimal conditions for the cultivation of the virus of spring-summer encephalitis are: infection of embryos of 9-10 days incubation by applying the virus on the chorionallantois membrane and incubating at 37C for 72 hours. During this the greatest quantity of virus is detected in the brain and body of the embryo, in the yolk and chorion-allantois membrane, the least- in the fluid and blood of the embryo.

4 Tables.

Table 1. Determination of the optimal period of incubation of infected embryos for the multiplication of the virus.

Period of incubation in hrs	Virus of Jap Encephalitis		Virus of Spring-Summer Enceph.	
	Tested material	LD50 for Mice	Tested material	LD50 for Mice
24 hrs	Suspension of tissues of foetus and membrane	10-4	—	—
48 hrs	Same	10-5	Suspension of tissue of foetus and membrane	10-3
72 hrs	Same	10-4.8	Same	10-6.3
96 hrs	Same	—	Same	10-6

Table 2. Influence of the age of the infected embryos on the cultivation of viruses.

Age of the embryo in days.	Above	Above	Above	Above
5	—	—	Suspension of tissue of foetus and membrane	10-4.7
7	Suspension of tissue of foetus and membrane.	10-4	Same	10-5
8	Same	10-5.5	—	—
9	Same	10-5.6	Same	10-6
10	—	—	Same	10-6.3
11	Same	10-6	Same	10-6.1
15	—	—	Suspension of brain	10-6.3

Table 3. Influence of temperature of incubation of the infected eggs on the accummulation of virus in them.

Temperature of incubation	Japanese Encephalitis Virus		Spring-summer Encephalitis Virus	
	Material tested	LD50 for mice	Material tested	LD 50 for mice
35.5 C	Suspension of tissue of foetus and membrane	10-5.2	Suspension of tissue of foetus & membrane	10-5.1
37. C	Same	10-5	Same	10-6
39. C	Same	10-2.2	Same	10-3.7

Table 4. Influence of path of infection of the embryos on the degree of multiplication of the virus.

Path of infection	See Above not tested	See Above LD50 mice	See Above not tested	See Above LD50 mice
On the chorionallantois membrane	Suspension of tissue of foetus & membrane	10-4.6	Suspension of tissue of foetus and membrane	10-5.8
In allantios cavity	Same	10-4.2	Same	10-4.4
In yolk sac	Same	10-5	Same	10-5.6
In amniotic cavity	---	---	Same	10-5.1

Journal of Microbiology, Epidemiology and Immunobiology USSR, No. 5, 1955
(Russian Ref. Zhur 56, No. 8, Item #30093)

Cultivating virus of spring-summer and Japanese encephalitis on developing chicken embryo, by O. G. Andzhaparidze

The most favorable conditions for the cultivation of the above virus have been established. For spring-summer encephalitis it is recommended that 9-11 day old embryos be infected in the chorio-allantois membrane, incubated 72 hours at 37°C. For virus of Japanese encephalitis - infect embryos, 8-9 days old, in the yolk sac, incubate 48 hours at 35.5. The greatest quantity of both viruses were detected in the brain of the embryo (10-6,7) and in its body (10-5). The quantity of virus injected has no effect on the regeneration.